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Fungal Contamination of Locally Processed Nigerian Food (Okpa): A Threat to Public Health

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Authors' contributions

This work was carried out in collaboration between both authors. Author PTN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PTN and APR managed the analyses of the study. Author PTN managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aim of this study is to determine the level of contamination associated with locally processed *Vignea subterranea* (okpa) flour in Nigeria.
Study Design: The completely randomized experimental design was adopted for this study.
Place and Duration of Study: The staff laboratory in University of Calabar Cross River State Nigeria was used for this research, within the space of two months.
Methodology: Standard microbiological research methods were adopted for the culture, isolation and identification of fungi. Spore heads and hyphae growth were compared with standard mycological atlas.
Results: Locally processed *Vigna subterranea* from South Eastern Nigeria markets and mills were analyzed for level of fungal contamination. *Aspergillus niger, Trychophyton spp, Penicillium spp, Rhizopus nigrican, Chrysosporium spp, Geotrichum spp, Mucor spp* and *Syncephalastrum spp* were identified at a percentage occurrence of 20.6, 5.6, 17.7, 20.6, 8.8, 2.9, 20.6 and 2.9 respectively. The Mean (x) percentage growth of fungal species was 12.46, a standard deviation (δ) of 8.19 and Variance (S.D)² of 67.08.
Conclusion: Locally processed *V. subterranea* in Nigeria can pose harm to the consumers of the

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food due to poor hygienic practice, unethical conduct and production inefficiency by food processors and handlers. Deliberate efforts must be made to curb the chances of contamination by microorganism especially fungi that may likely pose some level of resistance to heat.

Keywords: Locally; processed; Vigna subterranean; fungal; public; health; contamination.

1. INTRODUCTION

World Health Organization has recognized food contamination as a global issue [1,2]. This menace is more alarming in developing countries of the world [2]. Some African and Asian countries tend to suffer more food-borne epidemics caused by food contamination due to the low level of food safety measures. Serious food-borne disease outbreaks have reoccurred over time [3,4] in some Indian communities [5].

The demand for *V. subteranea* has increased over time in the local markets where producers sell them due to its appealing flavour. It has high nutritive value like other popularly consumed African legumes. This food is a delicacy in many West African countries. The South Eastern Nigerians are one of the highest consumers of bambara nut in Nigeria. It is most appreciated by the people when processed in the form of cake popularly referred to as Okpa [6,7]. Various communities have their peculiar ways of processing this nut for use. Grounding of seeds into flour is a major pattern for those who use it in the preparation of delicacies like soup, moi moi, akara, okpa etc. Freshly harvested pods may be cleaned up and boiled with salt and pepper for consumption. The black coloured bam nut is used by some communities for spiritual purposes since it is believed to possess some healing ability [8]. *V. subterranea* is nutritionally endowed. It contains reasonable amount of some major food classes such as protein, carbohydrate, and lipids. About 63% carbohydrate, 6.5% oil and 19% protein is present in *V. subterranea* seed [8]. No harm is associated with consumption of nut at different stages of maturity (so far it is well processed) [8,9,10].

V. subteranea is capable of growing in arid land where ground nut (*Arachis hypogeal*) maize *(Zea mays* L.) and sorghum *(Sorghum bicolar)* have failed [10]. Besides some Asian countries where *V. subteranea* is cultivated, it is rarely cultivated outside Africa. Its cultivation in Africa predates groundnut cultivation and well distributed in the Sub Saharan Africa (SSA). It is a highly recognized food by the culture of Western and Northern Cote d'ivorie [10]. Northern and South Eastern Nigeria value bambara nut as a traditional food [6,8]. The duration between the time of planting and maturity varies from one place to the other. The internal factors of a seed and external factors of the places where they are cultivated are believed to be a major contributor to the variability in the duration of maturity. It took 5 months for the seeds cultivated in Zaire to grow to maturity whereas; it took 3-4 months for seeds cultivated in Senegal and Madagascar to mature [8].

2. MATERIALS AND METHODS

2.1 Study Site and Sampling

The food markets of Enugu, Anambara, Ebonyi and Abia states of Nigeria were randomly sampled.

2.2 Sample Collection

Processed (flour) and unprocessed (nut) *Vigna suterranea* samples were collected from grinding mills and sellers of bambara nut in the markets with the aid of a clean plastic container. After collection, samples were stored in a clean covered container and kept in cool and dried environment for five to seven days. Afterwards, samples were transported to the laboratory for mycological analysis.

2.3 Culture and Identification

2.3.1 Media preparation

Titan Biotech Sabouraud Dextrose Agar (SDA) at a ratio of 65 g in 1000 ml of distilled water was prepared and sterilized in an autoclave at 121°c for 15 min. The medium was supplemented with amoxicillin a broad spectrum antibiotics at a concentration of 50 µg/1000 ml before pouring into sterile plates.

2.3.2 Total colony forming unit (cfu)

V. subterranea nuts were blended in a disinfected dry grain blender into fine particles

(flour) in the laboratory for control. One gram from each sample (both control sample and the locally processed samples collected from the markets) was separately dissolved in 9 ml of distilled water to form a solution in 15 ml test tube. Serial dilutions of the samples were done by pipetting 1 ml of the solution into a test tube with 9 ml of distilled water (labeled $x10^{-1}$). This procedure was repeated for subsequent tubes until it was diluted to a concentration of $x10^{-5}$. From each of the tubes, an aliquot of 0.1 ml was pipetted and spread on Sabouraud Dextrose Agar (SDA) in petri dishes with corresponding labels of dilution. Total fungal counts were determined after incubation at 28°c for 48 hours and after 96 hours.

2.3.3 Purification of isolates

Discrete colonies were randomly picked with aid of an inoculation loop and sub-cultured in SDA. The procedure was repeated until an absolute pure culture was obtained. The isolates were then stocked in SDA slant for further studies.

2.3.4 Slide culture

Slide cultures were prepared from 5-7 days from fungal pure cultures according to the methods adopted by [11].

2.3.5 Identification and characterization of isolates

Fungi identification was based on the macroscopic and microscopic morphology. Physiological characteristics ranging from the rate of growth, spore formation and colouration of culture were carefully studied. The stained slides from the slide culture were mounted and viewed using x40 objective lens on a microscope linked to the computer for appropriate morphological characteristics. Standard mycological atlas was used to identify the moulds. The resulting spore heads and mycelia were compared with existing pictures in the atlas.

2.4 Percentage Frequency of Fungal Occurrence

The percentage frequency of fungal occurrence in *V. subterranea* was determined by the formula stated below.

$$\frac{X}{Y} \times \frac{100}{1} = \% \text{ frequency}$$

Where:

X = Total number of each organism in a variety.
Y = Total number of all identified organism in a variety.

2.5 Moisture Content and pH of *V. subterranean*

2.5.1 Moisture content

The moisture content was determined using the formula shown below:

$$\text{Percentage Moisture content} = \frac{\Theta_2 - \theta_3}{\Theta_2 - \theta_1} \times \frac{100}{1}$$

Such that:

θ_1 = Weight of empty petri dish
Θ_2 = Weight of sample + petri dish before drying
θ_3 = Weight of sample + petri dish after drying

2.5.2 Measurement of pH

The glass electrode method was used to determine the pH of the food samples. A solution of the flour was prepared with deionized water according to standard procedures. Readings were taken when glass electrode was introduced into the sample solution.

2.6 Statistical Analysis

The following parameters were used to analyze the data generated in this research.

i) Mean $(\dot{X}) = \Sigma x/n$

ii) Mean Standard deviation $(\delta) = \frac{\sqrt{\Sigma x^2 - (\Sigma X)^2/n}}{n-1}$

iii) Variance $= (S.D)^2$

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Culture, isolation and identification of mould

Visible colonies of fast growing fungi were observed after 48 hours of incubation at room temperature as shown in tale one. Pure cultures of different moulds with varying morphological features were obtained. Blocks of pure cultures

were obtained with the aid of a cork borer and stained with lactophenol cotton blue solution. Afterwards, they were examined under phase contrast microscope (using ×40 objective lens) linked to the computer for appropriate microphotography. The resulting cultural morphology, spore heads and mycelia growth of *Aspergillus niger, Penicillium spp, Trichophyton spp, Chrysosporium spp, Mucor spp, Syncephalastrum spp Geotrichum spp and Rhizopus spp* are shown below.

Table 1. Mean viable counts of moulds isolated from *V. subterranea* (cfu/g)

No of days	$x10^{-1}$	$x10^{-2}$	$x10^{-3}$	$x10^{-5}$
Two days	–	8	-	2
Four days	24	14	1	22

Plate 1. Pure culture of *Mucor spp*

Plate 2. Pure culture of *Chrysosporium* spp

Plate 3. Pure culture of *Penicillium* spp

Plate 4. Pure culture of *Rhizopus nigricans*

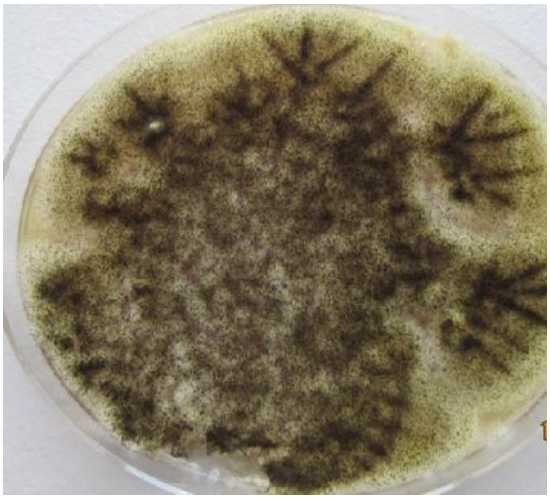

Plate 5. Pure culture of *Aspergillus niger*

Plate 6. Pure culture of *Trichophyton spp*

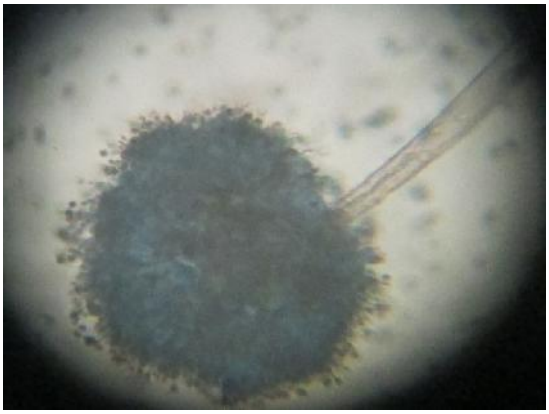

Plate 7. Spore head and mycelia of *Aspergillus niger*

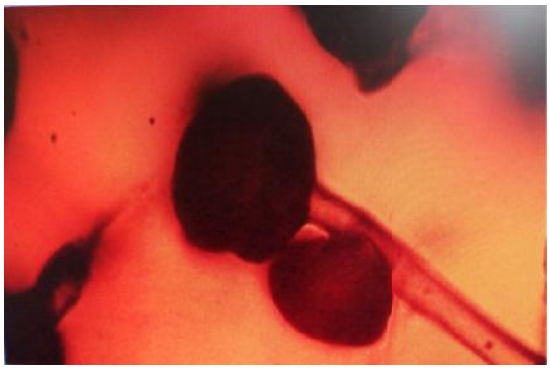

Plate 8. Micro morphology of *Rhizopus nigricans* (using x40 objective lense)

3.1.2 Fungal occurrence in control sample and locally processed *V. subterranea*

A total of eight genera of fungi were isolated from the control and the locally processed *Vigna subterranea* in this study. Excluding *Trichophyton spp, Syncephalastrum spp and Geotrichum spp* which were identified as contaminants of the flour alone as indicated in Table 2, the rest fungi isolated were contaminants of both the control and the processed food sample.

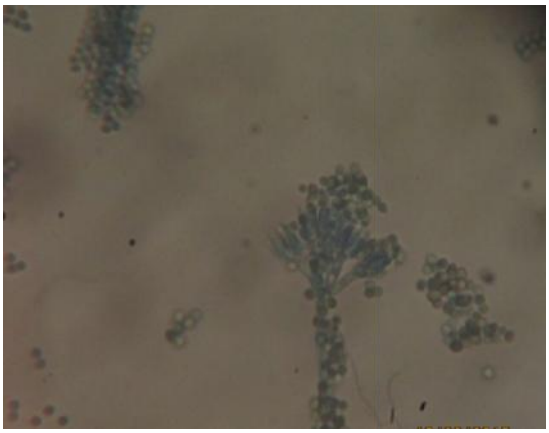

Plate 9. Micro morphology of *Penicillium species* (using x40 objective lense)

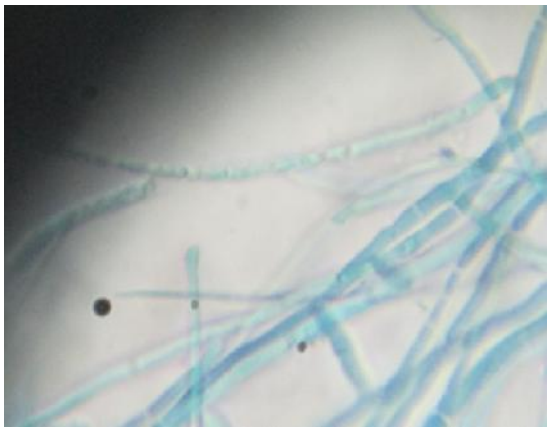

Plate 10. Microscopic morphology of *Geotrichum candidum*

Table 2. Distribution of fungal species in processed and unprocessed *V. subterranea*

Fungal species	Grain	Flour
Aspergillus niger	+	+
Trichophyton Spp	-	+
Geotrichum spp	-	+
Penicillium spp	+	+
Rhizopus nigricans	+	+
Chrysoporum spp	+	+
Mucor spp	+	+
Syncephalastrum spp	-	+

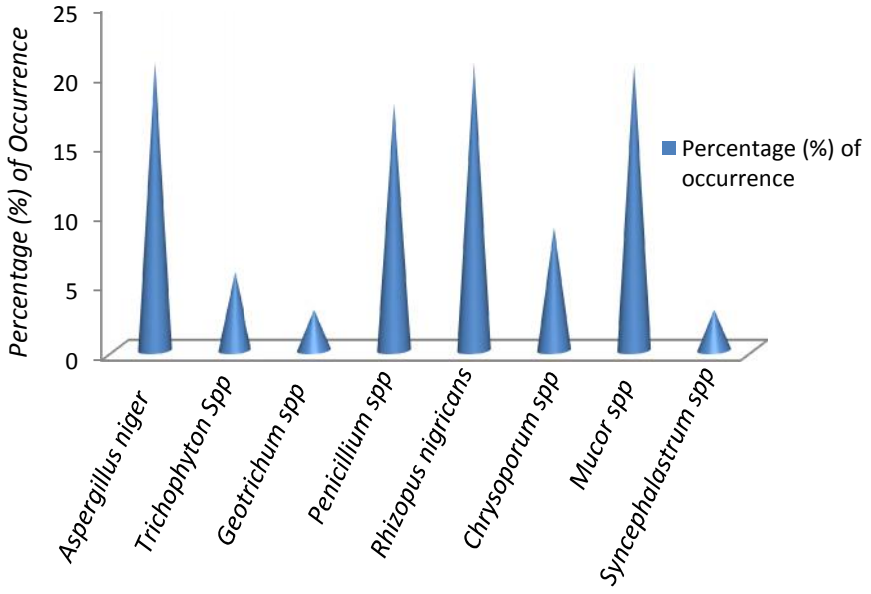

Fig. 1. Percentage occurrence of fungal contamination in locally processed *V. subterranea*

3.1.3 Moisture content and pH level of *V. subterranean*

The moisture content and pH of both samples (nut and flour) are shown in Table 3:

Table 3. Moisture content and pH level of *V. subterranean*

Varieties of *V. subterranea*	Percentage (%) moisture content	pH of samples
Control	3.48	7.06
Flour	16.99	7.01

3.1.4 Statistical analysis

3.1.4.1 Mean, standard deviation and variance of fungal growth

The mean growth of the fungi species is 12.46. The standard deviation of 8.19 and the variance in the fungal growth of 67.08 were obtained as shown below.

Mean ($\dot{X}$) = $\Sigma x/n$ = 12.46

Mean Standard deviation (δ) = $\frac{\sqrt{\Sigma x^2 - (\Sigma X)^2/n}}{n-1}$

= 8.19

Variance = $(S.D)^2$ = 67.08

3.2 DISCUSSSION

A total of eight species of fungi were isolated from *Vigna subterranea* in this study. *Aspergillus* and *Penicillium* species which were referred to as the most common storage fungi according to [12] were also major contaminants. *Aspergillus niger, Rhizopus nigrican and Mucor spp* in this study were the highest level of contaminant of *V. subterranea*. These moulds were also identified in the bare seed (control) and even in the locally processed flour. By this finding, it suggests that these moulds may have contaminated the food from the field (farm land) or possibly during storage and not necessarily during processing by handlers.

The isolation of *Trychophyton* species from the flour is most likely related to poor hygienic practice and production inefficiency by food handlers or processors. During sample collection, it was observed that bambara nut sellers who were sieving the grounded nut did so with some parts of their body exposed. This unethical conduct resulted in frequent contact of the processed food products with their cutaneous region, especially around their hand region as shown in Plate 11. It was also observed that the floor of the processing room where this food was being processed was not properly maintained as some parts of the floor were cracked. It is therefore very possible that *Geotrichum spp* may

have contaminated the processed food from the soil in the room. In addition, this fungus species may have as well contaminated the food sample through some droplets from their mouth while communicating since they failed to use mask to cover up their mouth and nose regions.

Plate 11. Poor handling and processing of *V. subterranea*

The contamination of *V. subterranea* by *Trychophyton, Geotrichum and Syncephalastrum* species is of public health significance. Such contamination of a popular choice food and delicacy of some Nigerians (especially in the North and South East of Nigeria) may result in the spread of Onychomycosis caused by *Trychophyton spp* and *Syncephalastrum spp* which also agrees with the finding of [13,14,15]. This food sample is capable of initiating disease conditions such as pulmonary geotrichosis, Brochial geotrichosis and Vaginal geotrichosis if they find their way to areas where they serve as opportunistic pathogens when other secondary handlers who are not properly protected come in contact with the food.

4. CONCLUSION

Locally processed *V. subterranea* in Nigeria can pose harm to the consumers of the food due to poor hygienic practices, unethical conduct and production inefficiency by food processors and handlers. Deliberate efforts must be made to curb the chances of contamination by microorganisms especially fungi that may likely pose some level of resistance to heat. Food handlers and processors should be properly dressed with all necessary wears as to ensure their safety and reduce contamination associated with droplets from the handlers. As much as possible, the food product must not have contact with the bare ground or soil after harvest from the farm, since the soil is the reservoir of most of the fungal contaminants.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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